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Redefinição do espectro clínico do Síndrome de Rett e Síndromes "Rett-like"

REDEFINITION OF THE PHENOTYPES OF RETT AND "RETT-LIKE" SYNDROMES

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Dissertação em Mestrado Integrado em Medicina

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LISTA DE ABREVIATURAS

CDKL5 - Cyclin-dependent kinase-like 5

EEG – Electroencephalography

FOXG1 - Gene forkhead box G1

GI - gastrointestinal

MECP2 - Methyl CpG binding protein 2

MRI - Magnetic resonance imaging

RTT – Rett Syndrome

Resumo

O Síndrome de Rett é uma doença do neurodesenvolvimento que afeta aproximadamente 1 em cada 10,000 indivíduos do sexo feminino. A mutação no gene methyl-CpG-binding protein 2, ligado ao X, é causadora do síndrome na maioria dos doentes. Para além desta mutação, mutações noutros genes têm vindo a ser associadas a formas atípicas: no gene forkhead box G1, envolvido na variante congénita do síndrome de Rett e no gene cyclin-dependent kinase-like 5 envolvido no Sindrome de Rett com encefalopatia epilética de início precoce.

Os critérios de diagnóstico do Síndrome de Rett foram pela última vez atualizados em 2010 por Neul et al. Após a revisão de um vasto número de relatos de caso e séries de casos publicados onde são descritos doentes com mutações em genes associadas às variantes de RTT, tentamos delinear uma relação genótipo-fenótipo para doentes com mutações nos genes forkhead box G1 e cyclin-dependent kinase-like. Este conhecimento tem aplicação na prática clinica dado que permite um refinado reconhecimento clínico da doença e dirigir o estudo genético de forma mais precisa para cada uma das variantes do Síndrome de Rett.

Por outro lado, com a definição de um fenótipo mais comum para cada uma das mutações, foi possível uma comparação com os critérios de classificação atuais para variantes de Síndrome de Rett. Concluímos, que poucos casos descritos como variantes cumprem os atuais critérios de diagnóstico para variante de Rett, e uma vez que se definem com características específicas e individualizantes podem possivelmente emergir como uma entidade independente.

Palavras-Chave: *Síndrome de Rett, Variante atípica do Síndrome de Rett, gene FOXG1, gene CDKL5, relação genótipo- fenótipo*

Abstract

Rett syndrome is a severe neurodevelopmental disease that affects approximately 1 in 10,000 live females and is caused, in the majority of the typical cases, by mutations in the X-linked methyl-CpG-binding protein gene. Mutations on other genes have also been associated with atypical variants of the Rett syndrome: in the forkhead box G1 gene - involved in the congenital variant – and in the cyclin-dependent kinase-like 5 gene - involved in the early-onset epileptic encephalopathy variant of the syndrome.

The diagnostic criteria of Rett Syndrome have been updated in 2010 by Neul et al. providing a key to diagnose of the Rett variants. Given the vast number of case reports of patients with mutations in the genes associated with the Rett Syndrome variants, we were able to refine the genotype-phenotype correlation for the forkhead box G1 gene and for cyclin-dependent kinase-like 5 gene. This knowledge has clinical application, given that it allows for a more refined recognition of the clinical picture and to more accurately target the genetic testing.

Through the analysis of a vast number of reported cases of each mutation we were able to build an approximate phenotype for each mutation, which in turn is essential for easier and faster recognition of the disease among physicians (and a valuable key in the decision of the performance of the genetic screening). On the other hand, taking into account a more broad phenotype, we compared the variants of Rett Syndrome with the current diagnostic criteria and verified that only few patients do fulfill the diagnostic criteria of a Rett variant. We concluded that mutations in these two genes and the respective associated phenotypes present specific characteristics, and may be considered as new independent entities.

Key Words: *Rett Syndrome, Atypical Rett Syndrome, FOXG1 gene, CDKL5 gene, genotype-phenotype correlation*

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Introduction

History of the disease - RTT and current diagnostic criteria

Rett syndrome (RTT) is a severe neurodevelopmental disease that affects approximately 1 in 10,000 live female births(1). This syndrome, originally described in the 1960's by Andreas Rett, is characterized by loss of spoken language and hand use with the development of distinctive hand stereotypies(2). The eponym Rett's Syndrome first appeared in 1983 when Hagberg wrote his first paper about the specific clinical features of this syndrome(3).

The gestation and birth are usually uneventful and head circumference at birth is normal(4). The first psychomotor anomaly appears during the first year of life, as a nonspecific slowdown in development. Patients rarely crawl and unsupported walk is usually delayed or never reached.

By the first or second year, loss of acquired fine motor, intellectual and communication abilities occurs: children lose interest in the environment and ability to speak, manipulate objects and play. Concomitantly they initiate repetitive movements with their hands and other body parts – stereotypies. The following stage, which can last from three to five years, is characterized by rapid regression. During this stage behavioral disturbances (unexpected screaming attacks, laughing spells and nocturnal insomnia) are common. Throughout the months of regression, stereotypies become more frequent and usually one pattern predominates (washing, clapping, wringing or tapping the hands). Autistic features may be evident (4).

Following this period of regression, there is frequently a phase of improvement of social skills, sometimes accompanied by a partial regain of lost abilities, such as the use of words. These girls remain profoundly intellectually disabled and present with a particularly good eye contact (4).

By this time, the combination of a previous personal history of developmental regression, deceleration of head growth, severe intellectual disability, continuous hand stereotypies, inability to use the hands and a striking good eye gaze suggest the diagnosis of RTT(5). The presence of other alterations such as bruxism, breathing dysfunction, peripheral vasomotor disturbances (hypotrophic small and cold feet)(6) and scoliosis favore the diagnosis. Epilepsy is also present in the majority of these children (up to 80%)(7). Seizures usually start during the third stage of the disease (around 5 years old) and become less frequent with aging

(8). As neuromotor functions slowly decline some parkinsonian signs such as, ataxia, limb wasting, dystonia, pyramidal signs, bradikinesia and rigidity may appear. (9).

The last stage is initiated when walkers lose the capacity of walking, or when patients who never acquired independent gait, are more than 10 years old. At this stage, hand stereotypies become less frequent faithful to its pattern (4).

RTT is quite underdiagnosed in adulthood, probably due to the much less exuberant stereotypies. By this time, patients are often wheelchair dependent. One third of the cases die, inexplicably, during the first two decades of life(4).

Some patients present with a core of symptoms of classical RTT, but show considerable variation in type and age of onset, severity of impairment and profile of clinical course. Consequently, RTT variants have been described (6, 10):

- Preserved speech variant (PSV): characterized by the recovery of speech to some extent;
- Congenital variant: without a normal period of development before regression;
- Early seizure variant: in which onset of seizures occurs before the regression;
- Forme fruste: with milder/incomplete clinical course (regression between 1 and 3 years).

Diagnostic criteria for RTT are periodically reviewed in order to clarify and simplify the diagnosis of this syndrome. Neul et al. (2010) (11) published the last Revised RTT Diagnostic Criteria for typical or classic RTT and for atypical RTT. According to these new criteria, history of regression followed by a period of recovery/stabilization is obligatory to establish the diagnosis of both typical and atypical RTT.

Revised Clinical Criteria for Typical RTT: Necessary criteria are now limited to the presence of regression plus four main criteria. The deceleration of head growth was eliminated from the necessary criteria because this feature is not found in all cases. However, it is still an important clinical sign that should raise the suspicion for the diagnosis. The supportive criteria have been entirely eliminated from the diagnostic criteria for typical RTT (Tab.1).

Revised Clinical Criteria for Atypical RTT: In addition to having regression, individuals must have at least two of the four main criteria and five of eleven supportive criteria (Table1).

Table 1: Revised Diagnostic Criteria for Rett Syndrome (RTT) *in* Neul et al (2010)

RTT diagnostic criteria 2010 Consider diagnosis when postnatal deceleration of head growth observed	
Required for typical or classic RTT	1. A period of regression followed by recovery or stabilization
	2. All main criteria and all exclusion criteria
	3. Supportive criteria are not required, although often present in typical RTT
Required for atypical or variant RTT	1. A period of regression followed by recovery or stabilization
	2. At least 2 of the 4 main criteria
	3. 5 out of 11 supportive criteria
Main criteria	1. Partial or complete loss of acquired purposeful hand skills
	2. Partial or complete loss of acquired spoken language
	3. Gait abnormalities: Impaired (dyspraxic) or absence of ability
	4. Stereotypic hand movements such as hand wringing/squeezing, clapping/tapping, mouthing and washing/rubbing automatisms
Exclusion criteria for typical RTT	1. Brain injury secondary to trauma (peri- or postnatally), neurometabolic disease, or severe infection that causes neurological problems
	2. Grossly abnormal psychomotor development in first 6 months of life
Supportive criteria for atypical RTT	1. Breathing disturbances when awake
	2. Bruxism when awake
	3. Impaired sleep pattern
	4. Abnormal muscle tone
	5. Peripheral vasomotor disturbances
	6. Scoliosis/kyphosis
	7. Growth retardation
	8. Small cold hands and feet
	9. Inappropriate laughing/screaming spells
	10. Diminished response to pain
	11. Intense eye communication - «eye pointing»

The frequencies at which the new supportive criteria are observed in atypical RTT were reported by Percy, et al. (2010)(11) : periodic breathing (61%), bruxism (83%), sleep disruption (72%), abnormal gait (93%), cold feet (19%), scoliosis (36%), aerophagia (38%), lower limb muscle atrophy (21%), laughing/screaming spells (63%), reduced nociception (71%) and intense eye contact (66%).

In 1999, Amir and colleagues discovered that mutations in the gene encoding Methyl-CpGbinding protein 2 (*MECP2*) are associated both with rare familial cases of RTT as well as with the more common sporadic occurrences of RTT (12). This gene is located in the long arm of the X chromosome, at band q28 and is subject to X-chromosome inactivation(13). The fact that mutations in this gene have been mainly identified in female patients, and the virtual absence of affected males, suggests an X-linked dominant inheritance pattern (3). As a matter of fact, females are heterozygous for mutations in *MECP2* gene and the few males reported have an XXY karyotype or *MECP2* mutations in a mosaic state.

In male patients the phenotype is wide and can range from a severe epileptic encephalopathy seen in patients who do have the *MECP2* mutations, to patients with a phenotype that resembles female patients with RTT (usually with mosaic form mutations or the Klinefelter syndrome) or even patients with a clinical picture that does not resemble RTT and have *MECP2* mutations infrequently found in female RTT patients(14, 15).

MECP2 mutations are present in classic RTT cases in 75% to 95% (11, 16-19) and only in 20-44% of the patients with RTT variants (17, 19, 20). However, Percy et al. (2010) (11) reported a higher incidence of mutations in RTT variants of 73%. It's worth mentioning that the mutations found in patients with RTT variant can be identical to those found in classic RTT. *MECP2* mutations have also been identified in about 50% of PSV cases and in lower percentage on the other variants (17).

Although it is a genetic disorder, 99.5% of RTT cases are sporadic(21). The *de novo* mutation often occurs on the paternal X chromosome, however, rare cases of germline mosaicism of *MECP2*(22) and of mildly affected or asymptomatic mothers (due to favorably skewed X-chromosome inactivation) passing on *MECP2* mutations occur, accounting for rare familial cases(14).

Rare individuals with mutations in *MECP2* present with other neurodevelopmental conditions such as autism(23), Angelman syndrome-like presentation(24), and non-specific intellectual disability; still they lack important defining features of the syndrome and the diagnosis cannot be made. These clinical phenotypes emphasize that mutations in *MECP2* are not synonymous of RTT and that a mutation in *MECP2* is not sufficient to make the diagnosis of RTT. Because *MECP2* mutations are neither necessary nor sufficient to make the diagnosis of RTT, RTT remains a clinical diagnosis(11).

There are significant clinical differences between RTT patients with and without *MECP2* mutation. Among those without the mutation, only few had a normal development during the first year of life and a regressive period was not evident; besides this, autistic behavior, when present, is noticed earlier. This suggests that these children may never have

been normal. Additionally, patients with no detected *MECP2* gene mutation have worse social contact and less stereotypies. Eye pointing and an ataxic-rigid gait seem to be important clues to the presence of mutation on the *MECP2* gene (19); these features may be of use in the decision of the necessity of a *MECP2* mutation screening.

The existence of RTT patients without *MECP2* mutations suggests that additional genetic factors might determine this disorder.

The congenital variant is one of the variants of atypical RTT, and, as said before, although up to 95% of classical RTT and 40% of atypical RTT are caused by mutations in the *MECP2* gene, only few girls described as congenital variants have mutations in *MECP2* (25, 26). Initially described by Rolando, the affected girls have an overlapping phenotype with classic RTT but in addition they were hypotonic and developmentally delayed from the very first months of life (27). In 2005, Shoichet et al. reported a 7-year-old girl exhibiting severe cognitive disability associated with a significant asymmetrical enlargement of the lateral ventricles, frontal and parietal myelination defects, complete agenesis of the corpus callosum, seizures, tetraplegia and microcephaly with a balanced *de novo* translocation t(2; 14)(p22;q12) with a neighboring inversion in chromosome 14q12 that disrupts the wingedhelix transcription factor forkhead box G1 (*FOXG1*) gene(28). Later, three 14q12 interstitial deletions encompassing *FOXG1* were detected in 2 girls with psychomotor retardation, epilepsy, microcephaly, and unusual facial features, and in a 10-month old male patient with intellectual disability, microcephaly, and facial dysmorphism (29-31). Finally, the importance of the *FOXG1* gene was reinforced by linking *FOXG1* –null mutations and the congenital variant of Rett syndrome (RTT) in 2 unrelated girls(32).

The most consistent RTT-like features observed in these patients were microcephaly, either of congenital onset or secondary to early postnatal deceleration of head growth, hand stereotypies, scoliosis, and some autonomic features including hypotrophic feet, bloating, and impaired nociperception. However, because RTT is a neurodevelopmental disorder affecting almost exclusively females, large molecular screening of the *FOXG1* gene were initially carried out in cohorts of female individuals suffering from typical and atypical forms of RTT(33-35), and this may explain why only few male patients have been reported to date with *FOXG1* point mutations (36).

The early seizure RTT variant was initially described by Hanefeld in 1985, who reported a girl with infantile spasms and hypsarrhythmia in early life (26, 37). This Hanefeld variant presents a phenotypic overlap with West syndrome and is also known as infantile spasm

syndrome, X-linked (ISSX). ISSX is characterized by the triad of infantile spasms, hypsarrhythmia, and severe to profound intellectual disability. In 2003, Kalscheuer et al. described 2 unrelated female patients with apparently balanced translocations: 46,X,t(X;7)(p22.3;p15) in 1 case and 46,X,t(X;6)(p22.3;q14) in the other(38). The 2 patients had a similar phenotype: severe early-onset infantile spasms with hypsarrythmia and profound global developmental arrest. In both patients, the X-chromosomal breakpoints disrupted *CDKL5*. In view of the phenotypic overlap between the Hanefeld variant and ISSX, 2 independent groups (in 2004) looked for *CDKL5* mutations in patients who had been diagnosed with RTT or a variant of RTT and in whom no *MECP2* mutation had been identified. They identified point mutations in the *CDKL5* gene in a subset of patients with a clinical picture resembling the early-onset RTT variant or with a history of early-onset seizures (39, 40). Noteworthy, the seizures observed in the affected individuals reported in these studies and those mentioned in other published reports of *CDKL5* defects (41) were generally difficult to control with anticonvulsant therapies. The *CDKL5* gene was subsequently analyzed in patients with both classic and atypical variants of RTT by several groups, but mutations were identified only in patients with seizure onset before 6 months of age (20, 42, 43).

Objectives

The purpose of this study is to gather a valuable number of articles with phenotypical descriptions of patients with either a mutation on *CDKL5* or *FOXG1*. We aim define a specific phenotype for each gene, that would allow physicians to use the clinical information to request targeted genetic testing

Methods

In order to review the highest number of case reports on RTT and its variants a systematic search was performed on MEDLINE. The selected *MESH terms* were: *human FOXG1 protein, human, human CDKL5 protein, Rett Syndrome, Zappella Variant, Atypical Rett Syndrome, Rett Syndrome, Preserved Speech Variant*. In addition, the reference lists of the selected papers were examined for additional studies. The search was not restricted by date and included articles until January 2013. We selected for studies providing clinical description of patients with mutations on *CDKL5* or *FOXG1*. We extracted all the clinical data in order to identify the required criteria for atypical or variant RTT.

For statistical analysis we used IBM® SPSS® Statistics Version 20.0 and descriptive analysis was conducted.

Results

We identified 53 citations in the initial search strategy, 6 were excluded for not being related with the issue, and 4 for providing only literature review. From the 43 articles considered to be relevant and selected for this review, 25 pertain to patients with *CDKL5* mutations, and 18 with *FOXG1*. The selected studies included 161 patients, 121 had mutations in *CDKL5* and 40 in the *FOXG1* gene.

Overall, 89,9% of the patients were females and 10,1% males (Table 2). Analyzing the *CDKL5* group, 93,4% of the patients were females and 6,6% males; the median age on the last evaluation was 5 years (minimum: 0,5; maximum: 41 years). On the other hand, on the *FOXG1* group, 78,9% were females and 21,1% males; the median age on the last evaluation was 4 years (minimum: 0,8; maximum: 41 years).

A period of **regression** followed by recovery or stabilization was found in 16,5% of the individuals with mutations in *CDKL5*, and in 22,5% of the patients with mutations in *FOXG1*.

Partial or complete **loss of acquired purposeful hand skills** was identified in 4,1 % of the individuals with mutations *in CDKL5*, and in 2,5% of the patients with mutations in *FOXG1* (Table 2). However the vast majority of the remaining patients did not even acquire purposeful hand skills.

Only 5% of the individuals in the *CDLK5* group showed partial or complete loss of **acquired spoken language**, while the rest never acquired language. None of the patients with *FOXG1* mutation ever acquired language.

Gait abnormalities, either impaired (dyspraxic) or absence of ability, were evident in 86,8% of the individuals in the *CDKL5* group, and in 90% of the patients in the *FOXG1* group.

Stereotypic hand movements such as hand wringing/squeezing, clapping/tapping, mouthing and washing/rubbing automatisms were described in 78,5% of the individuals with mutations in *CDKL5*, and in 87,5% of the patients with mutations in *FOXG1*.

In summary, 73,6% of the patients with *CDKL5* mutation and 77,5% of the patients with *FOXG1* mutation meet the two out of four main criteria necessary to establish the diagnosis of atypical RTT.

Considering the **supportive criteria** for diagnosis of the atypical RTT in the patients with mutations *in CDKL5*: 77,7% abnormal muscle tone, 25,6% peripheral vasomotor disturbances, 20,7% impaired sleep pattern, 19% presented breathing disturbances, 16,5% growth retardation, 14% scoliosis or kyphosis, 13,2% intense eye communication - «eye

pointing», 12,4% bruxism, 11,6% small cold hands and feet and 9,1% inappropriate laughing or screaming spells.

Considering the **supportive criteria** for diagnosis of the atypical RTT in the patients with mutations in *FOXG1*: 80% presented abnormal muscle tone, 37,5% small cold hands and feet, 35% peripheral vasomotor disturbances, 25% bruxism, 22,5% breathing disturbances, 20% growth retardation, 20% intense eye communication - «eye pointing», 17,5% scoliosis or kyphosis, 15% impaired sleep pattern, 10% inappropriate laughing or screaming spells. Diminished response to pain was not described in the majority of the articles included.

Only 7,4% of the patients with *CDKL5* mutation and 27,5% of the patients with *FOXG1* mutation meet the five out of the eleven supportive criteria.

Only 5% of the individuals with the *CDKL5* mutation and 2,5% of the individuals with the *FOXG1* mutation met the diagnostic criteria for RTT variant.

Diminished response to pain was not described in the majority of the articles reviewed.

Other clinical characteristics

All the *FOXG1* mutated patients presented with severe intellectual disability, while only 3% with *CDKL5* mutation did not have severe intellectual disability.

Regarding the presence of GI disturbances, they were evident in 15,7% of the *CDKL5* patients, and in 37,5% of the *FOXG1* patients. The most common were gastroesophageal reflux disease (GERD), constipation and dysphagia.

Feeding disturbances were more frequently described in patients with mutations in the *FOXG1* (47,5% vs 5,8% on the patients with *CDKL5* mutation).

Epilepsy was present in 98,3% of the *CDKL5* patients with a median age of onset of 1,5 months (minimum: 0,2; maximum: 11 months), and in 57,5% of the *FOXG1* patients with a median age of onset of 6 months (minimum: 4; maximum: 11 months). Infantile spasms were described in 57,0% of the *CDKL5* patients, and in 12,5% of the *FOXG1* patients. Refractory epilepsy was identified in 66,4% of patients, but data on 29% was not available. Only 7,5% of patients with *FOXG1* mutations were reported to have refractory epilepsy. EEG revealed hypsarrhytmia in 12% on the *CDKL5* group (13 from 108 pathological EEG patterns) and 7,6% (1 from 13 pathological EEG patterns).

Concerning the brain MRI (Magnetic Resonance Imaging), 36 out of 71 *CDKL5* patients who performed a MRI (50%) presented with abnormalities, the most common being frontal atrophy. On the other hand, 28 out of the 35 *FOXG1* patients (80%) who performed a MRI

presented with a brain abnormality the most common being *corpus callosum* hypoplasia/agenesia (Table 3).

Table 2 – Overall Results

	CDKL5	*FOXG1*
	% (N)	
Female gender	93,5% (113)	78,9% (30)
Severe intellectual disability	95,6% (109)	100% (40)
A period of regression followed by recovery or stabilization	16,5% (20)	22,5% (9)
1. Partial or complete loss of acquired purposeful hand skills	4,1%(5)	2,5% (1)
2. Partial or complete loss of acquired spoken language	5,0% (6)	0% (0)
3. Gait abnormalities: Impaired (dyspraxic) or absence of ability	86,8% (105)	90% (36)
4. Stereotypic hand movements such as hand wringing/squeezing, clapping/tapping, mouthing and washing/rubbing automatisms	78,5% (95)	87,5% (35)
At least 2 of the 4 main criteria	73,6% (89)	77,5% (31)
1. Breathing disturbances when awake	19% (23)	22,5% (9)
2. Bruxism when awake	12,4%(15)	25% (10)
3. Impaired sleep pattern	21,5% (26)	15% (6)
4. Abnormal muscle tone	77,7% (94)	80% (32)
5. Peripheral vasomotor disturbances	25,6% (31)	35% (14)
6. Scoliosis/kyphosis	14% (17)	17,5% (7)
7. Growth retardation	16,5% (20)	20% (8)
8. Small cold hands and feet	11,6% (14)	37,5% (15)

9.Inappropriate laughing/screaming spells	9,1% (11)	10% (4)
10. Diminished response to pain	1,7% (2)	0% (0) 100% Unknown
11. Intense eye communication - «eye pointing»	13,2% (16)	20% (8)
5 out of 11 supportive criteria	7,4%	27,5% (11)
Rett variant criteria accomplished	5% (6)	2,5% (1)

Table 3 – Cerebral MRI findings in patients with *CDKL5* and *FOXG1* mutation

	***CDKL5* (N)**	***FOXG1* (N)**
Performed Cerebral MRI	71	35
Pathologic findings	36	28
Cortical and subcortical atrophy	28	2
Myelination defects	-	7
Abnormal white matter signal	-	5
Corpus callosum hypoplasia/agenesia	2	20
Lateral ventricles asymmetry	2	-
Cortical dysplasia	1	-
Syringomielia	1	-
Dysplasic hippocampus	-	1
Right hippocampal lesion	1	-

Discussion

As mentioned before, according to the Neul et al (2010) diagnosis criteria, the diagnosis of a RTT variant is based on (see table 4):

1. A period of regression followed by recovery or stabilization
2. At least 2 of the 4 main criteria
3. 5 out of 11 supportive criteria

According to the new criteria most of the individuals previously diagnosed with a RTT variant, would, actually, not fit this diagnosis given that they do not meet all the necessary criteria to the diagnosis. **Only 5% (N=6) of the individuals with the *CDKL5* mutation and 2,5% (N=1) of the individuals with the *FOXG1* mutation met the diagnostic criteria for RTT variant.** Most of them did not present regression (83,5% of the *CDKL5* patients and 77,5% of the *FOXG1* patients), and even though a majority was described as presenting 2/4 of the main criteria (73,6% of the *CDKL5* mutated patients and 77,5% of the *FOXG1* mutated patients), an important percentage did not present with 5/11 of the supportive criteria, also necessary to the diagnosis (92,6% of the *CDKL5* mutated patients and 72% of the *FOXG1* mutated patients). However, it is important to take into account that most of the articles analyzed were published before the publication of these criteria. Therefore, they may not provide a description of all the criteria necessary to the diagnosis. In addition, the patients described may have been studied before regression ensued, or regression could have gone unnoticed.

Interestingly, two of the main criteria: "partial or complete loss of acquired purposeful hand skills" and "partial or complete loss of acquired spoken language", were rarely met since the majority of patients never acquired neither hand skills nor language. This raises the question on whether absence of acquisition of hand skills or language should be included in the criteria for the diagnosis of these patients, as it happens with the gait criteria ("Gait abnormalities: Impaired (dyspraxic) or absence of ability").

In addition, the high prevalence of epilepsy in patients with mutations in *CDKL5* or in *FOXG1* (less evident in the latter), raises the question of whether epilepsy should be considered as supportive or even essential criteria.

Regarding the limitations of our study: it is important to note that the lack of information on some of the required criteria for variant RTT diagnosis may lead to underdiagnosis of some of the cases (it could happen that the patients actually met the criteria

but the authors didn't identify or reported on them). Also, even though we tried to be as thorough as possible in the search for relevant articles, some relevant but unpublished reports may be missing. Nevertheless, this limitation might have been overcome taking into account the significant number of case reports included and the estimated low prevalence of *CDKL5/FOXG1* mutation syndromes.

Accurate analysis of these data allowed us to point out the most important and distinctive characteristics of the individuals with the *CDKL5* and *FOXG1* mutations.

CDKL5

According to the literature revised, epileptic encephalopathy is the core symptom of *CDKL5* -related disorders (44-47), which is in agreement with our results. In our analysis, the majority of the patients with *CDKL5* mutation had early-onset epilepsy episodes. In the literature, various epileptic phenotypes have been described, but a 3-stage evolution proposed by Bahi-Buisson et al (2008) summarized the evolution of the *CDKL5*-related epileptic encephalopathy (45): early epilepsy (stage 1), then infantile spasms (stage 2) and, finally, multifocal and refractory myoclonic epilepsy (stage 3). Infantile spasms and refractory myoclonic epilepsy are frequent but not always present in *CDKL5* mutation patients (20, 39, 40, 42-44, 46-65).

Almost all the patients showed hand stereotypies, the most frequent being hand mouthing and clapping. These movements usually appeared in the first year of life, but their evolution is unknown(51).

Rett-like neurovegetative symptoms such as breathing disturbances, cold extremities and gastrointestinal disturbances were not as common as they are in Classical RTT. Finally, childhood-onset scoliosis was uncommon in *CDKL5* mutation patients.

Cerebral nonspecific abnormalities were found in the majority of the patients with a pathologic MRI. Cortical atrophy (frontal atrophy was the most described feature) combined with hyperintensities in the temporal lobe white matter were the most common abnormalities.

The male patients described had a more severe encephalopathy and virtually no acquisition of motor skills compared with the *CDKL5* mutation females. Rett-like features have not been described in males with *CDKL5* point mutations (40, 57, 61, 66, 67).

FOXG1

Patients with *FOXG1* mutation are characterized by a normal delivery, followed by severe presentation, excluding the classic period of regression necessary to the diagnosis of

RTT. The early-onset deceleration of head growth progressively resulting in absolute microcephaly before 4 months of age is almost always seen. (1, 28, 30, 31, 68-70). According to the literature, a relative preservation of eye contact - though not as intense eye gaze as the eye pointing of classical RTT - is also a common feature, which was present in 20% of the patients analyzed.

According to our data, a large percentage of the patients had feeding difficulties and features of autonomic origin, such as cold and hypotrophic extremities and abdominal bloating. This is in agreement with previous studies, and also with the fact that more patients with *FOXG1* mutation had 5/11 supportive criteria (27,5% vs only 7,4% of the patients with *CDKL5* mutation). Thus, the supportive criteria are more commonly met in patients with mutations in *FOXG1*.

Patients showed intense hyperkinetic movement disorders with polymorphic midline stereotypies and jerklike movements mainly consisting of axial and limb myoclonia. Moreover, they had bruxism and repetitive protrusive tongue movements. Epilepsy is also a frequent feature (32), the EEG pattern does not suggest any specific epilepsy syndrome. In the vast majority of cases and in contrast with *CDKL5* -related disorders, seizures were easily controlled by antiepileptic drugs.

An elevated percentage of patients with *FOXG1* mutation presented a pathologic MRI. The most typical MRI pattern, according to our data and with the literature, is the hypoplasia/agenesia of the *corpus callosum*. Thus, this abnormality constitutes a key feature of *FOXG1* -related encephalopathy (34, 36).

Conclusion

In conclusion, according to the new criteria for diagnosis of RTT and RTT variants, only 5% of the *CDKL5* patients, and 2,5% of the *FOXG1* patients previously reported would meet the criteria for diagnosis of RTT variant. In addition, these patients share few key characteristics of RTT, and most lack the mandatory regression of development. Also, as many authors have already stated, these individuals present specific features, (1). Consequently, one should be open to the possibility that mutations in these two genes and the respective associated phenotypes may be considered as new independent entities. Still, it is important to take into consideration that some of the articles used were not very detailed in the description of the patients, not providing all the necessary information for the diagnosis using the new criteria, and also, that the patients described may have been studied before regression, or regression could have gone unnoticed (as mentioned before).

Finally, we should suspect that a patient has a mutation in the *CDKL5* gene if is female and has severe intellectual disability, gait abnormalities (impaired or absence), absence of hand skills and language, stereotypies (most hand mouthing and clapping) and refractory epilepsy starting at 1,5 months with infantile spasms.

Other common clinical characteristics that might help the diagnosis are: the presence of hypotonia and impaired sleep pattern. The finding of frontal atrophy documented on MRI may also be helpful.

We should suspect that a patient has a mutation in the *FOXG1* gene if it has an early global development delay or severe intellectual disability, gait abnormalities (impaired or absence) – grossly abnormal initially development and stereotypies, absence of hand skills and language.

Other common clinical characteristics that might help the diagnosis are: hypotonia, epilepsy, usually controlled with therapy; feeding disturbances, small cold hands and feet and peripheral vasomotor disturbances, GI disturbances, bruxism and breathing disturbances. In this context an abnormal MRI usually with *corpus callosum* hypoplasia/agenesia may also suggest *FOXG1* mutations.

Our study expands the current knowledge of this group of neurodevelopmental disorders since it provides original information that can help physicians to lessen the

unnecessary requisition of a genetic screening test for the *MECP2* mutation based on phenotype.

Although classical RTT has been described quasi-exclusively in girls, *FOXG1* and *CDKL5* - related mutations have been identified in both female and male patients, showing the importance of screening in both female and male patients with an emerging developmental profile suggestive of the mutation.

Our results support the concept that developing longitudinal studies is of paramount importance to uncover the natural history of patients with mutations either on *CDCKL5* or *FOXG1*.

Future reports of patients with the diagnosis of Rett variant according to the revised criteria from Neul et al., will certainly clarify *CDKL5* and *FOXG1* phenotypes. However, it is important that the authors are as thorough as possible and report on other comorbidities that may be helpful on completing the clinical picture of these disorders.

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